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ORIGINAL PAPER

Variability in seed traits and genetic divergence in a clonal seed orchard of *Dalbergia sissoo* Roxb.

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Abstract: The variations in seed and pod traits, genetic superiority and genetic divergence were evaluated for a Clonal Seed Orchard (CSO) of *Dalbergia sissoo* Roxb. at Bithmera, India consisting of 20 clones from different agro-climatic conditions of four northern states (Uttar Pradesh, Uttarakhand, Haryana and Rajasthan). The seeds and pods of various clones in the orchard exhibited significant variability in size, weight and other characters. Significant positive correlations were observed between seed length and seed width (p<0.05), seed length and seed thickness (p<0.01), seed length and seed weight (p<0.01), seed thickness and seed weight (p<0.01), seed length and germination value (p<0.05). The genetic parameters for seed and pod traits also showed a wide range of variations in the orchard. Heritability values were found to be over 50 percent for most of the seed and pod traits. Seed weight, seed length and seed thickness showed high heritability values coupled with maximum genetic gain for these characters. Ward's minimum variance dendrogram of clones of *D. sissoo* showed three distinct clusters; cluster 1 was the largest with 12 better clones whereas cluster 2 and 3 consisting of seven moderate clones and one poor clone, respectively. Mean cluster values showed sufficient variation among the clusters for seed weight, germination value and seed length. The possible hybridization between best clones of cluster 1 to the disease resistant clone of cluster 2 (resistant against deadly *Gandoderma lucidum* root rot disease of *D. sissoo*) is also suggested for further breeding programmes of the species. The deployment of clone 194 (better performed and disease resistant) is also recommended in future plantation programmes of *D. sissoo* in northern India.

Keywords: clones; *Dalbergia sissoo*; divergence; seed traits; variability

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Introduction

Dalbergia sissoo Roxb. is one of the important indigenous multipurpose tree species of northern India and occurs throughout the sub- Himalayan region from Indus river to Assam and Himalayan valleys up to an elevation of 1 400 m.a.s.l. with rainfall ranging from 750 to 4 500 mm. Natural forests of *D. sissoo* are common in sub- Himalayan tract either pure or mixed with *Shorea robusta*, *Acacia catechu*, *Terminalia tomentosa* (Champion and Seth 1968). The species exhibits considerable variation in form and growth characters in its natural range. Most of the variation seems to be a function of edaphic, altitudinal and associated climatic factors in the region. However, some variation seems to be under strong genetic control and might be of adaptive importance. Genetically controlled variation presents ample opportunities for genetic upliftment of tree species. Genetic variation for seed germination and seedling growth among the seed sources of *D. sissoo* has been recorded (Vakshasya et al. 1992; Rehman et al. 1994; Sagta and Nautiyal 2001). Clonal variation in growth, flowering and seed production of *D. sissoo* in one of the seed orchard at Dehradun was also reported by Nautiyal et al. (2003).

The breeding programme of forestry species included phenotypic mass selection in wild stands and the use of the material for seed production in clonal seed orchards. Seeds collected from these orchards are then used for quality seedling production for reforestation of natural stands. When the clones in a seed orchard originate from a wide geographic area, different patterns of geographic variation may be revealed for phenological traits. The geographic variation in individual tree species may reveal an underlying pattern of genetic variation shaped by natural selection under specific geoclimatic conditions and other evolutionary processes. The pattern of genetic variation is typically expressed by correlations between environmental variables and genetic source response (Morgen – Stern 1996). The genetic quality of the seeds depends on the genetic superiority of the plus trees, their relationships, their combination ability (display hybrid vig-

our when crossed), and the rate of pollen contamination, among other factors.

Genetic divergence is the process in which two or more populations of an ancestral species accumulate independent genetic changes (mutations) through time, often after the populations have become reproductively isolated for some period of time. In some cases, subpopulations living in ecologically distinct peripheral environments can exhibit genetic divergence from the remainder of a population, especially where the range of a population is very large. The genetic differences among divergent populations can involve silent mutations (that have no effect on the phenotype) or give rise to significant morphological and/or physiological changes. Species are formed when populations genetically diverge till they cannot interbreed, hence, continuing to evolve and diverge independently. The hierarchical grouping shows a relationship between entities that are maximally similar for specified characteristics. This technique of clustering involves measurements of the forces of differentiation at two levels namely inter and intra, and enables selection of genetically divergent parents for the tree improvement/hybridization programme. Hierarchical cluster analysis has been shown to be useful tool for management of the variation in germplasm collections; the variation in germplasm collections is used to access genetic similarity and dissimilarity.

The Forest Research Institute, Dehradun has selected plus trees of *D. sissoo* from the states of Uttar Pradesh, Uttarakhand Haryana and Rajasthan based on growth performance and established a clonal seed orchard at Bithmera, Hissar in the state of Haryana. Seeds of the orchard are collected every year and used as a source of quality seed for improved planting stocks in northern India. As such there was no information about seed variability and genetic parameters among various clones of this orchard. The genetic parameters are very useful tools in predicting the amount of gain expected from the seed orchards. The variation among the clones is commonly used as an estimate of total genetic variation and used to calculate the degree of genetic control for a particular trait. Further, such data are also needed to genetically upgrade the orchards by selective culling of inferior types. With this background this paper reports the variations present in seed and pod traits, genetic superiority and genetic divergence in the first generation of a clonal seed orchard of *D. sissoo* at the age of 10 years.

Material and methods

Seed orchard and data collection

The studied clonal seed orchard of *D. sissoo* is located at Bithmera, Hissar (29°33' N, 75°55' E and 220 m altitude) and was established in July 1998 and is now managed by the Haryana forest department adopting standard orchard techniques to encourage higher flowering and fruiting. The clonal seed orchard was planted following a randomized block design with more than 20 clones in three replications at spacing of 6 m × 6 m. The climate in the area is subtropical with an annual mean maximum and minimum temperature of 45.6°C and 1.3°C, respectively; with annual rainfall of 373.90 mm. The detail information of the clones present in the orchard is presented in Table 1.

Table 1. Passport data of *D. sissoo* clones of the orchard.

Clone	Source	State	Latitude (^{0}N)	Longitude (^{0}E)	Altitude (m)	Rainfall (mm)
12	Haridwar	Uttarakhand	29.58	78.13	357	986
19	Haridwar	Uttarakhand	29.58	78.13	357	986
20	Haridwar	Uttarakhand	29.58	78.13	357	986
57	Ambala	Haryana	30.21	76.52	262	1334
66	Yamunanagar	Haryana	30.30	74.60	253	1417
80	Shriganganagar	Rajasthan	27.00	74.00	175	311
86	Hanumangarh	Rajasthan	27.00	74.00	175	311
87	Hanumangarh	Rajasthan	27.00	74.00	175	311
90	Hanumangarh	Rajasthan	27.00	74.00	175	311
93	Lakhanwali	Rajasthan	27.00	74.00	225	311
94	Hanumangarh	Rajasthan	27.00	74.00	175	311
101	Hanumangarh	Rajasthan	27.00	74.00	175	311
192	Gonda	Uttar Pradesh	27.28	82.01	176	1019
194	Gonda	Uttar Pradesh	27.28	82.01	176	1019
198	Gonda	Uttar Pradesh	27.28	82.01	176	1019
199	Gonda	Uttar Pradesh	27.28	82.01	176	1019
252	Rishikesh	Uttarakhand	30.20	78.04	528	2136
255	Rishikesh	Uttarakhand	30.20	78.04	528	2136
262	Rishikesh	Uttarakhand	30.20	78.04	528	2136
266	Rishikesh	Uttarakhand	30.20	78.04	528	2136

Pods were collected from five trees of each of the 20 different clones of the orchard during February 2008. Collection trees were selected randomly from each block of the orchard. The pods were bulked and labeled separately clone wise, and then dried in the sun to extract seeds manually. Pods of each clone (100 pods replicated five times) were measured for pod length, pod width, number of seeds/pod. Extracted seeds of each clone (100 seeds replicated five times) were also measured for seed length, seed width, seed thickness, seed weight of 100 seeds and germination as per standard rules (ISTA 1993). Seed germination was carried out using 100 seeds of each clone in four replications, and seeds were enclosed in 9.5 mm Petri dishes on germinating papers that were kept moist with distilled water. These Petri dishes were placed in the germinator having temperature of 30°C. The seed was considered germinated when the radicle was about 1.0 cm long. Germination count was recorded daily and germination test was run for 21 days. The germination data were used to calculate germination value (GV) as per formula of Czabator (1962) which is as:

$$GV = MDG \times PV$$

where MDG is mean daily germination calculated as the percentage of seed germination at the end of the test divided by the number of days to the end of the test; PV is the peak value, or the maximum quotient derived from all of the cumulative seed germination percentages on any day divided by the number of days to reach this percentages.

Analysis of variability

The observations were computed for analysis of variance

(ANOVA) as per Sukhatme and Amble (1989).

Source of variation	Degree of freedom	Mean sum of square	Expectation of mean square
Replication	r-1 (r=5)	Ms_r	$\sigma^2e + r\sigma^2r$
Clone	c-1 (c=20)	Ms_c	$\sigma^2e + \sigma^2c$
Error	(r-1) (c-1)	Ms_e	σ^2e
Total	(rc-1)	-	-

where, c and r are the number of clones and replications, respectively

Variance: The genotypic and phenotypic components of variance were calculated from ANOVA as described by Burton (1952).
Genotypic variance: $(\sigma^2g) = \sigma^2c - \sigma^2e)/r$
where σ^2c = clone mean squares, σ^2e = error mean squares and r = number of replication
Phenotypic variance: $(\sigma^2p) = \sigma^2g + \sigma^2e$
Genotypic coefficient of variance: GCV= $\surd\sigma^2g$/Mean ×100
Phenotypic coefficient of variance: PCV = $\surd\sigma^2p$/Mean ×100
Heritability: Broad sense heritability was calculated as per Lush (1994).
Heritability (h^2) = σ^2g /σ^2p ×100
Genetic Advance: The genetic advance was calculated as per Johnson et al. (1955).
Genetic Advance (Gs) = $K.h^2 \surd\sigma^2p$
where K is selection differential (2.06 at 5% selection intensity (Cotterill and Dean, 1990)
Genetic gain: The expected genetic gain, in percent of mean, was calculated following (Burton and Devane, 1993).
Genetic gain = Gs ×100/ Mean

Genetic divergence

Genetic divergence of different clones was measured by Ward's grouping method (Ward 1963a and b). Clustering of clones was done on the basis of three important characters viz. seed weight, seed length (both have high heritability values) and germination value (good indicator of seed quality). The clustering develops a relationship between clones that are maximally similar for specified characters. Each of the clones is genetically different from another. However, the extent of difference might vary to a certain degree. Thus, in clustering number of clusters may vary from one to the total number of clones used.

The collected data on various aspects were analyzed statistically for variability, genetic superiority and divergence using SPSS package (Anon 2007).

Results and discussion

Seed variability

The seed and pod parameters showed significant differences at 5% level between the clones of the orchard (Table 2). Maximum and minimum seed length of 8.60 mm and 5.18 mm was recorded in clone 94 and 20, respectively. Maximum seed width of 4.71 mm was also recorded in Clone 94 and minimum 3.14 mm in clone 80. Maximum seed thickness was observed for clone 80, while minimum by clone 93 of the orchard. Seed weight varied from 0.62 g (clone 20) to 1.71 g (clone 94) in the orchard. Seed thickness and pod width exhibited higher range of variability than other traits. Maximum seed weight in clone 94 of Rajasthan origin (region with comparatively less rainfall) and minimum in clone 20 from Haridwar (region with more rainfall) verifies Wright's (1976) concept that seed weight in forest trees is generally higher in drier areas than in wetter areas.

Variability in seed characters was also observed in clones in clonal seed orchard of *Santalum album* (Annapurna et al. 2005) and in seeds of the selected plus trees (Bagchi and Sharma 1989) of the same species in south India. Different seed sources of *D. sissoo* also exhibited such significant variations in seed traits (Singh and Pokhriyal 2001). Mamo et al. (2006) reported the variation in seed and germination characteristics among *Juniperous procera* populations in Ethiopia.

Details of correlation coefficients for different parameters like seed length, seed width, seed thickness, seed weight, number of seeds per pod, pod length, pod width, germination value are presented in Table 3. Significant positive correlations were observed between seed length and seed width ($p < 0.05$), seed length and seed thickness ($p < 0.01$), seed length and seed weight ($p < 0.01$), seed thickness and seed weight ($p < 0.01$). In pod parameters, pod width shows a positive correlation with seed width and pod length with number of seeds per pod. Vakshasya et al. (1992) and Gera et al. (2000) also found significant positive correlations between seed, pod morphometric traits while studying different seed sources of *D. sissoo*.

Seed size, especially seed weight is regarded as an important aspect of reproductive strategy as it plays a key role in the establishment of the juvenile phase of life cycle. A number of selective advantages with large seed size have been documented, such as prolonged dormancy during unfavourable light conditions, development of large amounts of photosynthetic tissue, allowing quick seedling growth, and dispersal modes (Harms et al. 2000; Leishman et al. 2000). The significant positive correlation between seed length and germination value ($p<0.05$) in the present study suggests that seed size have some importance in predicting the seed quality in *D. sissoo* and seed morphometric traits might not be under selection pressure for other purposes like seed dispersal etc.

Genetic variability

The estimates of phenotypic and genotypic variances as well as the coefficient of phenotypic and genotypic variation of different characters are shown in Table 4. The relative amount of variation in different characters can be correlated by comparing the coefficient of phenotypic and genotypic variation of each character. In general, both the coefficient of phenotypic and genotypic variation was of comparable magnitude for all the characters. The heritability estimates (broad sense) were found highest for seed weight (0.90) followed by seed length (0.89), seed width (0.74) and seed thickness (0.52). In pod characters, heritability was 0.70 for pod width followed by pod length (0.57) and number of

seeds/pod (0.37). The expected genetic gain by selecting the 5% best as percent of mean was found to be maximum for seed weight (43.70) followed by seed thickness (25.21) and seed length (23.97). Seed weight, seed length and seed thickness provide a sufficient amount of genetic variability as it is evidenced from GCV and PCV estimates in combination with heritability and genetic gain. Johnson et al. (1955), Singh and Uppal (1977), Volkar et al. (1990), Singh and Chaudhary (1993) reported that heritability estimates along with estimates of expected genetic gain are more useful than the heritability itself in predicting the resultant effect for selecting the best genotypes for a given trait.

Table 2. Seed and pod parameters of different clones of *D. sissoo* (Mean ± Standard Deviation).

Clone	Seed length (mm)	Seed width (mm)	Seed thickness (mm)	Seed weight (g)	Pod length (cm)	Pod width (cm)	No. of seeds per pod
12	6.36± 0.26	3.63± 0.21	0.51± 0.052	1.01± 0.025	4.50±0.42	0.44± 0.17	1.36± 0.17
19	5.69± 0.18	3.45± 0.34	0.27±0.049	0.91±0.034	4.90±0.48	0.54±0.05	1.40±0.24
20	5.18± 0.12	3.51± 0.13	0.34± 0.030	0.62± 0.017	4.64±0.30	0.62±0.08	1.84± 0.31
57	7.37±0.18	4.17± 0.15	0.65±0.030	1.02±0.017	4.16±0.17	0.50± 0.03	1.32± 0.26
66	7.21± 0.33	3.92± 0.12	0.71± 0.053	1.31± 0.059	3.98± 0.33	0.84± 0.05	1.40± 0.24
80	7.97± 0.22	3.14± 0.14	1.00± 0.036	1.45± 0.031	4.48± 0.42	0.48± 0.04	1.44± 0.33
86	7.54± 0.21	4.43± 0.13	0.59± 0.021	1.47± 0.053	4.90± 0.63	0.52± 0.08	1.64± 0.33
87	6.99± 0.13	3.69± 0.16	0.59± 0.015	1.29± 0.053	4.98± 0.16	0.56± 0.05	1.24± 0.22
90	6.09± 0.28	3.77± 0.15	0.43± 0.033	0.88± 0.058	4.36± 0.38	0.52± 0.08	1.48± 0.33
93	6.72± 0.30	4.03± 0.19	0.25± 0.024	0.98± 0.022	5.50± 0.55	0.60± 0.07	1.72± 0.30
94	8.6± 0.28	4.71± 0.23	0.64± 0.047	1.71± 0.066	4.26± 0.22	0.84± 0.08	1.32± 0.18
101	6.38± 0.52	3.95± 0.24	0.42± 0.039	1.52± 0.193	4.30± 0.28	0.72± 0.04	1.56± 0.17
192	5.70± 0.13	3.51± 0.19	0.42± 0.013	1.16± 0.177	5.12± 0.69	0.74± 0.13	2.24± 0.68
194	6.79± 0.28	3.73± 0.11	0.52± 0.031	1.43± 0.034	5.16± 0.55	0.6± 0.12	2.16± 0.26
198	5.59± 0.24	3.51± 0.14	0.36± 0.024	1.05± 0.158	4.36± 0.33	0.48± 0.04	1.84± 0.40
199	6.56± 0.28	3.61± 0.23	0.42± 0.030	1.01± 0.057	5.50± 0.19	0.54± 0.05	1.80± 0.32
252	6.27± 0.40	4.02± 0.40	0.43± 0.064	1.26± 0.150	4.44± 0.44	0.50± 0.07	1.40± 0.28
255	6.55± 0.16	3.86± 0.17	0.55± 0.042	1.17± 0.037	4.40± 0.16	0.54± 0.05	1.28± 0.22
262	6.25± 0.25	4.12± 0.09	0.46± 0.055	1.01± 0.014	5.56± 0.30	0.66± 0.05	1.52± 0.23
266	6.81± 0.40	4.24± 0.28	0.46± 0.045	0.99± 0.038	5.32± 0.57	0.88± 0.04	1.52± 0.36
CV	12.58	9.68	33.97	22.78	10.33	21.98	17.90
SE	0.17	0.13	0.02	0.06	0.24	0.05	0.19
CD (0.05)	0.34	0.25	0.04	0.11	0.48	0.10	0.38

Note: CV = Coefficient of Variation, SE = Standard Error, CD = Critical Difference

Table 3. Correlation coefficient of seed, pod and vigour traits in *D. sissoo*.

Characters	Seed length	Seed width	Seed thickness	Seed weight	Pod length	Pod width	No. of seeds per pod	Germination value (GV)
Seed length	1.000	0.512*	0.754**	0.727**	-0.194	0.211	-0.414	0.477*
Seed width		1.000	0.014	0.366	-0.031	0.452*	-0.289	0.328
Seed thickness			1.000	0.574**	-0.405	0.009	-0.364	0.245
Seed weight				1.000	-0.262	0.250	-0.123	0.423
Pod length					1.000	0.072	0.444*	0.041
Pod width						1.000	0.095	-0.126
No. of seeds per pod							1.000	-0.067
Germination value (GV)								1.000

* Correlation is significant at 0.05 level (2 – tailed); ** Correlation is significant at 0.01 level (2- tailed)

Table 4. Variance, coefficient of variation, heritability and genetic gain in *D. sissoo*.

Characters	Phenotypic variance	Genotypic variance	GCV	PCV	Heritability	Gs	G. Gain
Seed length	0.74	0.66	12.29	13.00	0.89	1.58	23.97
Seed width	0.17	0.12	9.29	10.74	0.74	0.63	16.55
Seed thickness	0.03	0.02	18.78	25.92	0.52	0.30	25.21
Seed weight	0.074	0.067	24.03	25.25	0.90	0.50	43.70
Pod length	0.36	0.02	9.64	12.63	0.57	0.71	15.02
Pod width	0.02	0.01	21.08	25.05	0.70	0.21	36.49
No. of seeds per pod	0.15	0.05	15.48	25.26	0.37	0.30	20.31

Where GCV = Genotypic coefficient of variance, PCV = Phenotypic coefficient of variance, Gs = Genetic Advance, G. Gain = Genetic Gain

The estimates of variability with regard to genetic parameters for seed traits in this study showed a wide range of variation. The variation among clones is commonly used as an estimate of total genetic variation and to calculate the degree of genetic control for a particular trait (Foster and Shaw 1988). Heritability values were found over 50 percent for most of traits studied except number of seeds per pod. Seed weight, seed length and seed thickness showed high heritability values coupled with maximum genetic gain. Traits with such values indicate presence of good amount of heritable additive components and are under strong genetic control. The results of the present study also showed the fact that the high heritability did not always mean high genetic gain. It happens due to non- additive gene effects. The characters like seed weight, seed length and seed thickness had a very high genetic gain together with high heritability, indicating that high heritability obtained in these characters is due to additive gene effects (Panse 1957; Misra and Saini 1988).

Genetic divergence

Ward's minimum variance dendrogram of clones of *D. sissoo* showed three distinct clusters based on three characters viz. seed length, seed weight and germination value. The distances among the clusters indicated how entities are fused together (agglomerated) (Fig. 1). Cluster 1 was the largest cluster consisting of 12 clones (12, 252, 255, 262, 80, 86, 94, 101, 192, 194, 198 and 199) followed by cluster 2 with seven clones (266, 19, 87, 90, 93, 57 and 66) and cluster 3 with only one clone (20). The mean values of seed length, seed weight and germination value of respective clusters are presented in Table 5. Mean cluster values showed sufficient variation among the clusters for seed length, seed weight and germination value as these characters were showed significant variations at 5% level between the clones (Table 2). Cluster 1 had the highest values for three traits viz. seed weight (1.27 gm), germination value (94.46) and seed length (6.71 mm) followed by cluster 2 and cluster 3. Clone 20 of cluster 3 was differentiated from other clones of cluster 1 and 2 having more genetic distance as seen from Fig. 1.

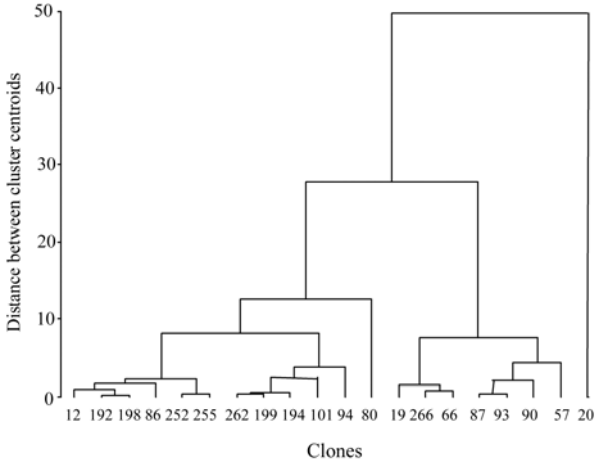

Fig. 1 Ward's minimum variance dendrogram (CSO Bithmera, Haryana)

Table 5. Cluster mean of the characters of *D. sissoo* of the orchard.

Cluster number	Cluster means		
	Seed weight	Germination value	Seed length
1	1.27	94.46	6.71
2	1.05	66.77	6.69
3	0.62	34.57	5.18

Though the cluster analysis grouped the genotypes (clones) with greater similarity for seed characters, they did not necessarily include the genotypes from the same source of origin. The results with Hierarchical Ward's method of grouping of clones in this orchard indicated that distribution of genotypes into different groups did not follow any pattern with regard to source of origin. Cluster 1 consisted clones having origin from three states i.e. Uttarakhand (12, 252, 255, 262), Rajasthan (80, 86, 94, 101) and Uttar Pradesh (192, 194, 198, 199), whereas cluster 2 also consisted clones originated from three states Uttarakhand (19, 266), Rajasthan (87, 90, 93) and Haryana (57, 66). However, cluster 3 has only one clone 20 from Uttarakhand origin. Abdelmahmound and Obeid (2010) also indicated that distribution of genotypes into different groups did not follow any defined pattern with regard to the country of origin, while studying sugarcane genotypes in Sudan. The comparative study of 20 clones indicated that clones from cluster 1 had the highest values for all the three traits viz. seed weight, germination value and seed length followed by cluster 2 and cluster 3 with minimum values. In this way, best, moderate and poor clone fall in cluster 1, 2 and 3, respectively. Determination of cluster behavior and genetic distance would help to maintain genetically diverse population of high yielding clones and using the material from such clones, it is possible to develop a breeding programme with selected individuals of desired constitution.

Genetic divergence studies have also been extensively carried out to determine the genetic divergence in provenance (Bagchi 1992) and progeny trials (Singh and Chaudhary 1992). Bhatt (1973) selected parents for hybridization programme using four different techniques and compared the performance of hybrids; the author found that the genetic divergence analysis was the most effective tool to identify the parents for hybridization. In another study in the same orchard, Harsh (2007) found clone 194 and 266 as resistant clones against deadly *Gandoderma lucidum* root rot disease of *D. sissoo* and clone 94 and 101 as susceptible ones for the same disease. This study also concludes that clones 94, 101, 194 (cluster 1) performed better and clone 266 (cluster 2) as moderate one. Hence, there is a possibility to develop hybridization programme between best clones (94,101) from cluster 1 and the most resistant and divergent clone (266) of cluster 2. The present study also recommended the deployment of clone 194 (better performed and disease resistant) of *D. sissoo* in future plantation programmes in northern India.

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